REMARKS

The Office Action and the cited and applied reference have been carefully reviewed. No claim is allowed. Claims 93, 95, and 98-120 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

Claims 93, 95, 96 and 98-119 have been rejected under 35 U.S.C. §112, first paragraph, as lacking adequate written description. Although applicants do not concede to the examiner's position, this rejection is obviated by the amendment to claim 93 in order to advance prosecution.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 93, 95, 96 and 98-119 have been rejected under 35 U.S.C. §112, first paragraph, because the examiner states that the specification, while being enabling for claims limited in scope to a monoclonal antibody specifically recognizing a polypeptide of SEQ ID NO:2, wherein Xaa is Met or Thr, does not reasonably provide enablement for claims to monoclonal antibodies to variants of SEQ ID NO:2 or any "interferon-γ inducing protein". As in the above written description rejection, although applicants do not concede to the examiner's position, this rejection is obviated by the amendment to claim 93 in order to advance prosecution.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 95 and 95-120 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Nakamura et al., *Infect. Immun.* 61:64-70 (1993). The examiner still considers that "factor" disclosed in Nakamura's first 1993 publication is the same as the polypeptide of the present invention in view of Nakamura's later publication (*Infect. Immun.* 63:3966-3972, 1995). The examiner asserts that it would have been obvious for a skilled person to obtain an antibody against the "factor". This rejection is respectfully traversed.

Applicant has previously pointed out various differences between the polypeptide and the "factor" disclosed in Nakamura's first publication (1993) to show that they are different. A table summarizing the differences is provided below:

	Polypeptide of the present invention	"factor" of Nakamura's first publication
origin	liver of mouse	serum of mouse
molecular weight	19,000 ± 5,000 Da (SDS-PAGE)	50,000 ~ 55,000 Da (SDS-PAGE)
activity	will not lose its activity when treated with SDS-PAGE	will not lose its activity when treated with SDS-PAGE
IFN-γ inducing ability	being capable of inducing IFN-γ in IFN-γ producing cells with the polypeptide alone	being incapable of inducing IFN-γ in IFN-γ producing cells with the "factor" alone, but being capable of inducing IFN-γ in the presence of IL-2, Con-A, or anti-CD3 Mab (see Nakamura's first publication, p. 67, the second paragraph "Dual requirement and effect of a dose")

With regard to molecular weight, it should be noted that Nakamura's first publication states at page 69, left column, lines 3-5:

> The cytokines IL-1, IL-3, IL-4, IL-5, IL-6, and tumor necrosis factor, whose molecular weight are much lower than that of the factor…

On the other hand, as shown in "THE CYTOKINE Facts Book", attached hereto for the examiner's consideration, the cytokines IL-1, IL-3, IL-4, IL-5 and IL-6 have molecular weights in the range of about 17,000 to 50,000. Therefore, the molecular weight of 50,000 to 55,000 Da of the "factor" recited in Nakamura's first publication, page 66, right column, third line from the bottom, is consistent with the statement above. It is therefore clear and apparent that the molecular weight of the "factor" is 50,000 to 55,000 (SDS-PAGE).

By contrast, the polypeptide of the present invention has a molecular weight of 19,000 ± 5,000 Da (SDS-PAGE). In general, it is well recognized by those of skill in the art that an identical substance would never show different molecular weights when measured using the same method. Nevertheless, the examiner maintains that the polypeptide of the present invention is the same substance as the "factor" of Nakamura's first publication.

Applicants note that the examiner considers the "factor" disclosed in Nakamura's first publication as being the same as the polypeptide of the present invention in view of Nakamura's later publication. Nakamura's later publication reports:

> The IFN-γ producing cells in this case were NK cells, while it was unlikely that LPS directly stimulated NK cells (5). In our previous studies, an interleukin-12 (IL-12)-like, 75 kDa IFN-γ-inducing factor (IGIF) was observed in the sera of mice treated with *Propionibacterium acnes* challenged with LPS (16, 17). *In this study, we isolated an 18- to 19-kDa IGIF from these mice and characterized it. The serum factor whose apparent molecular weight mass was previously found to be 75 kDa by gel filtration was shown to contain the same 18- to 19-kDa IGIF.* (emphasis added; see page 3966, left column, second line from the bottom to right column, first paragraph) (Please note that reference publication "16" cited above is Nakamura's first 1993 publication.)
>
> Previously, we have shown that some unidentified factor with IL-18-like activities was released into the circulation systems of bacterium-treated, LPS-challenged mice (16, 17). *In this study, we have confirmed that a similar IFN-γ-inducing factor exists in the liver extracts of such mice (Fig. 1).* This factor was purified from the liver extract and analyzed for its molecular characteristics (Fig.2 to 4). *The molecular mass of the factor was about 18 to 19 kDa when estimated from both molecular sieving and SDS-PAGE.* Its isoelectric point determined by Mono P column chromatography was 4.8. The amino acid sequence of the NH2-terminal portion of this protein was determined and shown to be a novel protein. (emphasis added; see page 3970, right column,

> 6 line form the bottom to page 3971, left column, first paragraph).

One of ordinary skill in the art reading the above disclosures would reasonably consider that Nakamura's later publication does not assert that the "factor" of Nakamura's first publication is the same as the "factor" of Nakamura's later publication, but rather suggests that the "factor" of Nakamura's first publication contains "the factor having the molecular weight of 18 to 19 kDa" disclosed in Nakamura's later publication.

Applicants find it unreasonable to hold that the "factor" of Nakamura's first publication is the same as the "factor" of the later publication even if the later "factor" is contained in the former. Furthermore, applicants would like to draw the examiner's attention to Dr. Haruki OKAMURA's declaration filed on September 28, 2005. Dr. OKAMURA is one of the authors of Nakamura's first publication. In paragraph 10 of the declaration, Dr. OKAMURA states as follows:

> ... we speculated that the factor could possibly be natural killer stimulatory factor (NKSF)/interleukin-12 (IL-12)

Please note that "natural killer stimulatory factor (NKSF) interleukin-12 (IL-12)" has IFN-γ inducibility (please see paragraph 11 of the declaration).

This statement in the declaration of Dr. OKAMURA clearly shows that one of the authors of Nakamura's first publication *per se* did not even consider the "factor" to be the polypeptide with a molecular weight of 19,000±5,000.

In addition, applicants wish to draw the examiner's attention to the difference in specific activities. As shown in Table 1 at page 66 of Nakamura's first publication, the specific activity of the "factor" of the first publication after purification with Phenyl-Sepharose was "283,333 U/mg". By contrast, as shown in Table 1 at page 3969 of the later publication, the specific activity of the "factor" of the later publication (after the same purification with Phenyl-Sepharose) was only "14 U/mg". Please also note that the maximum specific activity of the "factor" of the later publication was "6,600 U/mg". If the "factor" of the first publication is the same as the "factor" of the later publication, then the specific activities of the both factors should be same after the same purification procedures. However, this is not the case. Furthermore, since the later publication employs higher purification procedures than the first publication, the maximum specific activity of the "factor" of the later publication would be expected by those of skill in the art to be higher than that of the "factor" of the first publication. Clearly, this is also not the case.

From the evidence above, one of ordinary skill in the art would reasonably conclude that the "factor" of the first Nakamura publication is not same as the "factor" of the later Nakamura publication. Accordingly, the presently claimed invention simply cannot be made obvious by Nakamura.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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THE CYTOKINE *FactsBook*

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THE CYTOKINE *FactsBook*

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Physicochemical properties of IL-1α and IL-1β

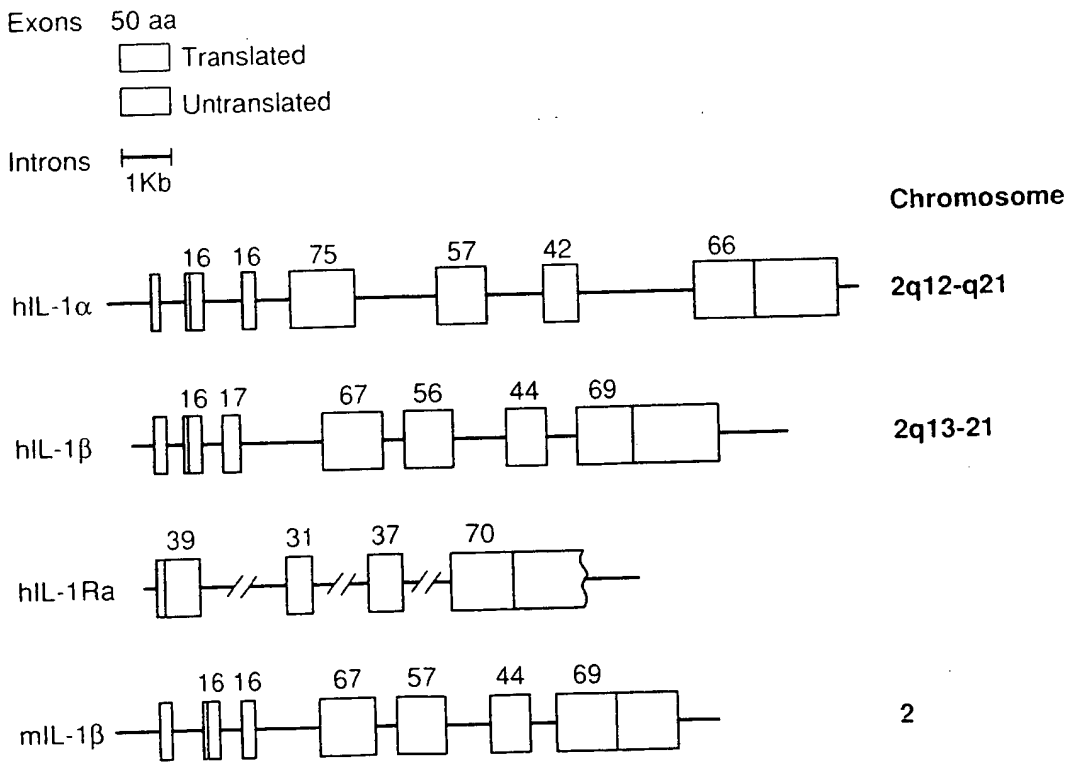
Property	IL-1α		IL-1β	
	Human	Mouse	Human	Mouse
pI	5	5	7	7
Amino acids – precursor	271	270	269	269
– mature[a]	159	156	153	159
M_r (K) – predicted	18.0	18.0	17.4	17.4
– expressed	17.5	17.4	17.3	17.5
Potential N-linked glycosylation sites[b]	2	3	1	2
Disulphide bonds	0	0	0	0

[a] After proteolytic removal of propeptide
[b] IL1 is not normally glycosylated

3-D structure

The structure of IL-1α has been determined at a resolution of 2.7 Å by X-ray crystallography and IL-1β at lower resolution by NMR spectroscopy. *9,10* Both forms of IL-1 are stable tetrahedral globular proteins formed by an antiparallel six-stranded barrel closed at one end by a six-stranded β-sheet to form a bowl-like structure.

Gene structure *11–14*



Mouse IL-1α, IL-1β and IL-1Ra genes are all on chromosome 2, and have a very similar structure to the human genes.

IL-3

Other names

Mast cell growth factor (MCGF), multi-colony stimulating factor (multi-CSF), eosinophil-CSF (E-CSF), haematopoietic cell growth factor (HCGF), burst-promoting activity (BPA), P-cell stimulating factor activity, thy-1 inducing factor, WEHI-3 growth factor.

THE MOLECULE

Interleukin 3 is a haematopoietic growth factor which stimulates colony formation of erythroid, megakaryocyte, neutrophil, eosinophil, basophil, mast cell and monocytic lineages [1]. IL-3 may also stimulate multipotent progenitor cells, but it is more likely to be important in committing progenitor cells to a differentiation pathway rather than self-renewal of primitive stem cells. Many of the activities of IL-3 are enhanced or depend upon co-stimulation with other cytokines. IL-3 does not stimulate lymphocyte colony formation, but it is a growth factor for B lymphocytes and it activates monocytes, suggesting that it may have an additional immunoregulatory role. IL-3 has been used clinically to expand haematopoietic precursors after bone marrow transplantation, aplastic anaemia and chemotherapy [2].

Crossreactivity

Amino acid sequence homology between mouse and human IL-3 is only 29% and there is no cross-species reactivity.

Sources

Activated T cells, mast cells, eosinophils.

Bioassays

Proliferation of TF-1 (human erythroleukaemia), MO7e (human megakaryoblastic leukaemia) or AML-193 (acute myeloid leukaemia) cell lines. Stimulation of erythroid, granulocyte and macrophage colony formation in bone marrow colony assay.

Physicochemical properties of IL-3

Property	Human	Mouse
pI	4–8	4–8
Amino acids – precursor	152	166
– mature[a]	133	140
M_r (K) – predicted	15.1	15.7
– expressed	14–30	28
Potential N-linked glycosylation sites	2	4[b]
Disulphide bonds	1	2

[a] After removal of predicted signal peptide.

[b] Glycosylation only at positions 16 and 86 (see sequence). Glycosylation is not required for biological activity.

IL-4

Other names
B Cell stimulating factor 1 (BSF-1).

THE MOLECULE

Interleukin 4 is a pleiotropic cytokine derived from T cells and mast cells with multiple biological effects on B cells, T cells and many non-lymphoid cells including monocytes, endothelial cells and fibroblasts. It also induces secretion of IgG1 and IgE by mouse B cells and IgG4 and IgE by human B cells. The IL-4-dependent production of IgE and possibly IgG1 and IgG4 is due to IL-4-induced isotype switching [1–3]. IL-4 appears to share this property with IL-13.

Crossreactivity
Two regions of human IL-4 (amino acids 1–90 and 129–149) share approximately 50% sequence homology with the corresponding regions of mouse IL-4. In contrast, the region from amino acid positions 91–128 shares very little homology with the corresponding region of mouse IL-4. There is no cross-species reactivity between human and mouse IL-4.

Sources
Mast cells, T cells, some mouse B cell lymphomas, bone marrow stromal cells.

Bioassays
Human: Proliferation of PHA T cell blasts in the presence of blocking anti-IL-2 or anti-IL-2R antibody; proliferation of MO7 cell line; increased expression of CD23 or surface IgM on human tonsillar B cells.

Mouse: Proliferation of CTLL in the presence of anti-IL-2 or anti-IL-2R antibody. Increased expression of MHC class II on murine B cells.

Physicochemical properties of IL-4

Property	Human	Mouse
pI	10.5	6.5
Amino acids – precursor	153	140
– mature[a]	129	120
M_r(K) – predicted	15.0	13.6
– expressed	15–19	15–19
Potential N-linked glycosylation sites	2[b]	3
Disulphide bonds	3	3

[a] After removal of signal peptide.
[b] Asn38 is glycosylated.

IL-5

Other names
Eosinophil differentiation factor (EDF), eosinophil colony stimulating factor (E-CSF), B cell growth factor II (BCGFII), B cell differentiation factor for IgM (BCDFμ), IgA enhancing factor, T cell replacing factor (TRF).

THE MOLECULE

Interleukin 5 is a T cell-derived glycoprotein which stimulates eosinophil colony formation and is an eosinophil differentiation factor in humans and mice. It is also a growth and differentiation factor for mouse but not human B cells [1–3].

Crossreactivity
There is 71% homology between mouse and human IL-5 and significant crossreactivity in functional assays.

Sources
Mast cells, T cells and eosinophils.

Bioassays
Human: Eosinophil differentiation; proliferation of TF1 cell line.

Mouse: Eosinophil differentiation; proliferation of BCL1 or B13 B cell lines.

Physicochemical properties of IL-5

Property		Human	Mouse
pI		?	?
Amino acids	– precursor	134	133
	– mature[a]	115	113
M_r (K)	– predicted	13.1	13.1
	– expressed[b]	45	40–50
Potential N-linked glycosylation sites		2	3
Disulphide bonds[c]		2	2

[a] After removal of predicted signal peptide.
[b] Homodimer.
[c] Interchain.

3-D structure
IL-5 is an antiparallel disulphide-linked homodimer. The monomer is biologically inactive. The structure of the dimer has been determined at a resolution of 2.4 Å [4]. It has a novel two-domain structure with each domain showing significant structural homology to the cytokine fold in GM-CSF, M-CSF, IL-2, IL-4 and growth hormone. The IL-5 structure is made up of

Other names
Interferon-β2 (IFNβ2), 26-kDa protein, B cell stimulatory factor 2 (BSF-2), hybridoma/plasmacytoma growth factor (HPGF or IL-HP1), hepatocyte stimulating factor (HSF), monocyte granulocyte inducer type 2 (MGI-2), cytotoxic T cell differentiation factor and thrombopoietin.

THE MOLECULE

Interleukin 6 is a multifunctional cytokine secreted by both lymphoid and non-lymphoid cells which regulates B and T cell function, haematopoiesis and acute phase reactions [1–3].

Crossreactivity
There is 42% homology between mouse and human IL-6. Human IL-6 is functional on mouse cells but mouse IL-6 has no activity on human cells.

Sources
IL-6 is made by lymphoid cells (T cells, B cells), and many non-lymphoid cells including macrophages, bone marrow stromal cells, fibroblasts, keratinocytes, mesangium cells, astrocytes and endothelial cells.

Bioassays
Proliferation by IL-6-dependent B9 cell line. Increased Ig secretion by CESS or other EBV-transformed human lymphoblastoid B cell lines.

Physicochemical properties of IL-6

Property	Human	Mouse
Amino acids – precursor	212	211
– mature[a]	183[b]	187
M_r (K) – predicted	20.8	21.7
– expressed	26	22–29
Potential N-linked glycosylation sites	2	0[c]
Disulphide bonds[d]	2	2[d]

[a] After removal of predicted signal peptide.
[b] N-Terminal amino acids of human IL-6 derived from a T cell line, an osteosarcoma cell line and a liposarcoma cell line are Pro, Ala and Val respectively, indicating heterogeneity in the signal peptide cleavage site.
[c] There are several potential O-linked glycosylation sites.
[d] Potential disulphide bonds between Cys43–49, 72–82 in man and 46–52, 75–85 in mouse. These four Cys residues are conserved between human and mouse IL-6, G-CSF, LIF and CNTF.

3-D structure
Not known.